MODEL: (3PS32) Subcutaneously-Implanted P388 Leukemia

Origin of Tumor Line: Chemically induced in 1955 in a DBA/2 mouse by painting the skin with 3-methylcholanthrene.[1]

Summary of Test Procedures: 1×10^6 cells in ascitic fluid are implanted s.c. in CD2F1 mice. I.p. test agent treatment starts one day after tumor implant and is continued daily for a total of five injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

ANIMALS: (refer to Protocol 8)

Propagation: DBA/2 mice (CD2F1 for one generation if DBA/2 are not available).

Testing: CD2F1 mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

EXPERIMENT SIZE: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

Titrations: Each control is to include titrations of 1×10^7 to 1×10^3 cells, inclusive, with ten animals per level.

TUMOR TRANSFER: (refer to Protocols 2, 5, and 6)

Propagation:

Suspension: Prepare a suspension of diluted ascitic fluid so that a 0.1 ml portion contains 1×10^6 cells.

Time: Day 7.

Site: Implant i.p. 0.1 ml of suspension containing 1×10^6 cells.

Testing:

Suspension: Prepare a suspension of diluted ascitic fluid so that a 0.1 ml portion contains 1×10^6 cells.

Time: Day 7.

Site: Implant s.c. 0.1 ml of suspension containing 1×10^6 cells into axillary region.

TESTING SCHEDULE: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials. Test positive control compound in every experiment. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Randomize and weigh animals. Treat as instructed.

Day 2: Recheck cultures. Discard experiment if contaminated.

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[1]American Journal of Pathology, 33:No. 3, pp. 603, 1957.

Day 8: Toxicity day. Weigh animals and record.
Day 9: Control early-death day.
Day 22: Control no-take day.
Day 30: End and evaluate experiment.

QUALITY CONTROL: (refer to Protocol 7)

Schedule the positive control compound (NSC 8806* at doses of 4 and 2 mg/kg/injection) in every experiment, the regimen for which is i.p. QD 1-5. The lower T/C limit for the positive control is 150%. The acceptable untreated control median survival time is 13-16 days.

EVALUATION: (refer to Protocol 11)

The parameter measured is median survival time. Compute mean animal body weights for Day 1 and Day 8, compute T/C for all test groups with > 65% survivors on Day 8. A T/C value of < 86% indicates toxicity. An excessive animal body weight change difference (test minus control) may also be used in evaluating toxicity.

CRITERIA FOR ACTIVITY:

An initial T/C $\geq$ 125% is considered necessary to demonstrate moderate activity. A reproducible T/C $\geq$ 150% is considered significant activity.

REPORTING OF DATA:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or a special request) and for poor suspensions.

*Positive control compound NSC 8806 is Melphalan. CAS RN is 3223-07-2.